UNIVERSITI PUTRA MALAYSIA

EFFECT OF MICROBIAL ANTAGONISTS ON THE DEVELOPMENT OF BACTERIAL WILT ON TOMATO

CHOO EE FONG

FP 2003 5

EFFECT OF MICROBIAL ANTAGONISTS ON THE DEVELOPMENT OF BACTERIAL WILT ON TOMATO

By

CHOO EE FONG

Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia, in Fulfilment of the Requirement for the
Degree of Master of Agricultural Science

March 2003

Specially Dedicated

To

My Family

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement of the degree of Master of Agricultural Science

EFFECT OF MICROBIAL ANTAGONIST ON THE DEVELOPMENT OF BACTERIAL WILT ON TOMATO

By

CHOO EE FONG

March 2003

Chairman : Assoc. Prof. Dr. Hiryati Abdullah

Faculty : Agriculture

Bacterial wilt caused by *Ralstonia solanacearum* is the major constraint to the production of tomato in Malaysia. In this study, the effect of application methods of biological control agents to control bacterial wilt of MT11, a moderately resistant variety of tomato was evaluated under greenhouse and field condition. Six antagonistic strains of *Pseudomonas aeruginosa* showed *in vitro* antagonism towards *R. solanacearum*. Seed bio-priming with these strains of *P. aeruginosa* either individually or in combination increased the percentage of seed germination. Whereas, biomass of plants was increased only after seeds were bio-primed with the combined strain. Combination of strains KT8+72PTT was used as seeds bio-prime agent in greenhouse and field experiments.

Penicillium sp. showed *in vitro* antagonism towards *R. solanacearum*. Greenhouse experiment also showed that the antagonist could reduce the incidence of bacterial wilt on MT11. An experiment was carried out on the effect of the application methods and levels of concentration of the antagonist

on the biomass of plants. Results showed that soil incorporated with *Penicillium* sp. just before transplanting significantly reduced the biomass of top part of six true leaves seedlings. Direct drenching of antagonist also significantly reduced the biomass of four-week-old seedlings, either at high or low concentration. However, no significant reduction was observed in the biomass of root system and dry weight of top when the antagonist was incorporated into soil one week prior to transplanting.

Two greenhouse trials were conducted to evaluate the integrated control using combination of bacterial and fungal antagonist and calcium nitrate, on MT11. Both the experiments showed reduced incidence of bacterial wilt on plants, which were treated with the integrated control measures. Combined treatment of seed bio-priming and carrier application of *Penicillium* sp. at high concentration recorded the lowest Disease Severity Index of bacterial wilt in the second greenhouse trial. Carrier application of *Penicillium* sp. provided better protection to plants compared to direct drenching regardless of inoculum concentration.

In the field experiment, disease incidence on MT11 was reduced after combined treatment of seed bio-priming and application of *Penicillium* sp. directly or in carrier with calcium nitrate as supplement. Subsequently, tomato yield also increased by 129.6% and 133.4% as compared to the control after five harvests when treated with combined treatment of seed bio-priming with direct application of *Penicillium* sp. or in carrier application, respectively. However, the same integrated control measures did not provide sufficient protection to the susceptible variety, Pearl, which recorded 100% infection at

the end of experiment. With the susceptible variety, the integrated control measures significantly reduced disease incidence only at the third week after transplanting.

In conclusion, bio-priming of tomato seeds with *P. aeruginosa*, strains KT8 and 72PTT, with application of *Penicillium* sp. at transplanting stage was able to control bacterial wilt and thus increased the yield. This study indicated that timing and application method for microbial antagonists as biocontrol agents were critical.

Abstrak tesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Master Sains Pertanian

KESAN ANTAGONIS MIKOB TERHADAP PERTUMBUHAN PENYAKIT LAYU PADA TOMATO

OLEH

CHOO EE FONG

March 2003

Pengerusi : Prof. Madya Dr. Hiryati Abdullah

Fakulti : Pertanian

Penyakit layu bakteria yang disebabkan oleh *Ralstonia solanacearum* merupakan halangan utama kepada pengeluaran tomato di Malaysia. Dalam kajian ini, kesan penggunaan kaedah kawalan biologi untuk mengawal layu bakteria pada tomato MT11, varieti sederhana resistan terhadap penyakit ini telah dinilai di rumah kaca dan ladang. Enam *Pseudomonas aeruginosa* strain menunjukkan sifat antagonis terhadap *R. solanacearum* secara *in vitro*. Rawatan bijibenih secara biologi dengan *P. aeruginosa* strain ini secara tunggal atau dalam kombinasi, telah meningkatkan peratus percambahan bijibenih. Manakala, biojisim tumbuhan hanya dapat dipertingkatkan selepas rawatan biologi dengan kombinasi strain. Kombinasi strain KT8 + 72PTT dipilih sebagai agen rawatan biologi dalam kajian rumah kaca dan ladang.

Penicillium sp. menunjukkan sifat antagonisnya terhadap *R. solanacaerum* secara *in vitro*. Eksperimen rumah kaca juga menunjukkan antagonis ini dapat mengurangkan insiden layu bakteria pada MT11. Eksperimen telah dijalankan untuk mengaji kesan kaedah aplikasi dan tahap

kepekatan bagi antagonis pada biojisim tumbuhan. Keputusan menunjukkan bahawa pengaulan tanah dengan *Penicillium* sp. sebaik sebelum pemindahan tumbuhan telah mengurangkan biojisim bagi anak benih yang mempunyai enam daun sebenar. Pencurahan *Penicillium* sp. secara terus juga menurunkan biojisim bahagian atas bagi anak benih yang berumur empat minggu, sama ada pada kepekatan yang tinggi atau rendah. Walaubagaimanapun, tiada pengurangan yang bererti diperhatikan dalam biojisim sistem akar dan jisim kering bahagian atas anak benih, apabila antagonis dimasukkan dalam tanah seminggu sebelum pemindahan tumbuhan.

Dua ujian rumah kaca telah dijalankan untuk mengaji kawalan gabungan dengan kombinasi antagonis bakteria dan kulat serta kalsium nitrat ke atas MT11. Keuda-dua ujian ini telah menunjukkan pengurangan insiden layu bakteria dengan rawatan gabungan tersebut. Kombinasi rawatan bijibenih secara biologi, serta aplikasi *Penicillium* sp. oleh pembawa dalam kepekatan yang tinggi mencatatkan indeks keterukan penyakit yang terendah bagi layu bakteria dalam ujian rumah kaca yang kedua. Aplikasi *Penicillium* sp. dalam pembawa memberi perlindungan yang lebih baik kepada tumbuhan berbanding dengan cara pencurahan terus tanpa mengira kepekatan inokulum.

Eksperimen di ladang menunjukkan insiden penyakit pada MT11 berkurangan setelah dirawat dengan kombinasi rawatan bijibenih secara biologi serta aplikasi *Penicillium* sp. secara pencurahan atau dalam pembawa, dengan kasium nitrat sebagai tambahan. Seterusnya, hasil tomato

juga telah meningkat sebanyak 129.6% dan 133.4% berbanding kepada kawalan selepas lima pungutan, apabila dirawat dengan kombinasi rawatan bijibenih secara biologi serta aplikasi *Penicillium* sp. secara terus atau dalam pembawa, masing-masing. Walaubagaimanapun, ukuran kawalan gabungan yang sama tidak memberi perlindungan yang cukup kepada varieti rentan, Pearl, yang telah mencatatkan 100% jangkitan pada akhir eksperimen. Dengan varieti rentan, ukuran kawalan itu mengurangkan insiden penyakit dengan bererti hanya pada minggu ketiga selepas pemindahan tumbuhan.

Kesimpulannya, rawatan bijibenih secara biologi dengan *P. aeruginosa*, strain KT8 dan 72PTT dengan aplikasi *Penicillium* sp. pada peringkat pemindahan tumbuhan dapat mengawal layu bakteria dan turut menambahkan hasil. Kajian ini menunjukkan bahawa masa dan kaedah aplikasi adalah kritikal untuk antagonis mikrob berfungsi sebagai agen kawalan biologi.

ACKNOWLEDGEMENTS

First and foremost, I would like to dedicate my sincere appreciation to Associate Professor Dr. Hiryati Abdullah, Chairperson of the Supervisory Committee, for her constant guidance, encouragement, advice and remarkable patience in this project. My sincere gratitude is also dedicated to my supervisory committee members, Dr. Halimi Mohd. Saud and Professor Mohd. Ghazali Mohayidin, for their guidance, suggestions and supports in this study.

I would like to extend my acknowledgements to all the staffs in Microbiology Lab and Pathology Lab for their assistance, especially to Puan Zunaina for her care and kindness. Also to all others who have attributed and involved one way another to the successful completion of the project, they are conferred my sincere appreciation.

My heartfelt thanks are also dedicated to my friends, especially Neo and Kam Loong for their assistance and advice. Also to Shan, Teng, Siew Eim, Kak Nor, LC, Khairul, Phua, Sue and Chee Kin for their precious friendships, assistance and kindness.

My specially thanks also goes to my housemates, Peng Kong, Chin Peng, Siaw San, Mei Chooi, Weai Gean and Vincent, for their care and assistance for all these years.

Finally, I would like to express my heartiest gratitude and appreciation to my beloved parents, brothers, sister and Chee Siong for their unrelenting love and understanding throughout the years of my study. To Chee Siong, thanks for constant moral support for bringing me strength whenever I need them the most.

I certify that an Examination Committee met on 17th March 2003 to conduct the final examination of Choo Ee Fong on her Master of Agricultural Science thesis entitled "Effect of Microbial Antagonist on the Development of Bacterial Wilt on Tomato" in the accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follow:

Sariah Meon, Ph.D.,
Professor,
Department of Plant Protection,
Faculty of Agriculture,
Universiti Putra Malaysia.
(Chairman)

Hiryati Abdullah, Ph.D.,
Associate Professor,
Department of Plant Protection,
Faculty of Agriculture,
Universiti Putra Malaysia.
(Member)

Halimi Mohd. Saud, Ph.D.,
Lecturer,
Department of Land Management,
Faculty of Agriculture,
Universiti Putra Malaysia.
(Member)

Mohd. Ghazali Mohayidin, Ph.D.,
Professor,
Department of Agribusiness and Information System,
Faculty of Agriculture,
Universiti Putra Malaysia.
(Member)

GULAM RUSUL RAHMAT ALI, Ph.D.
Professor/Deputy Dean,
School of Graduate Studies,
Universiti Putra Malaysia.

Date: 16 JUN 2003

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Agricultural Science. The members of the Supervisory Committee are as follows:

Hiryati Abdullah, Ph.D.,
Associate Professor,
Department of Plant Protection,
Faculty of Agriculture,
Universiti Putra Malaysia.
(Chairperson)

Halimi Mohd. Saud, Ph.D.,
Lecturer,
Department of Land Management,
Faculty of Agriculture,
Universiti Putra Malaysia.
(Member)

Mohd. Ghazali Mohayidin, Ph.D.,
Professor,
Department of Agribusiness and Information System,
Faculty of Agriculture,
Universiti Putra Malaysia.
(Member)

AINI IDERIS, Ph.D.
Professor /Dean,
School of Graduate Studies,
Universiti Putra Malaysia.

Date: 11 JUL 2003

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that is has not been previously or concurrently submitted for any other degree at UPM or other institutions.

CHOO EE FONG

Date: 14 JUN 2003

TABLE OF CONTENTS

		Page
DEDICATION		ii
ABSTRACT		iii
ABSTRAK		vi
ACKNOWLEDEMENTS		ix
APPROVAL SHEETS		xi
DECLARATION FORM		xiii
LIST OF TABLES		xvii
LIST OF FIGURES		xviii
LIST OF ABBREVIATIONS		xxi
CHAPTER		
I	**INTRODUCTION**	1
II	**LITERATURE REVIEW**	4
	Tomato Plant Culture	4
	Plant Characteristics	7
	Bacterial Wilt	7
	The Disease	7
	The Pathogen- *Ralstonia solanacearum*	10
	Disease Symptom	13
	Factors Affecting Disease Dissemination and Development	15
	Survival of the Pathogen	15
	Environmental Factors	16
	Alternative Hosts	19
	Dissemination	20
	Control Measures	21
	Crop Rotation	21
	Resistant Variety	23
	Soil Amendments	24
	Role of Calcium	24
	Other Methods of Control	28
	Biological Control	28
	Introduction	28
	Fluorescent Pseudomonads	31
	Seed Treatment with *Pseudomonas fluorescens*	34
	Penicillium sp.	37
III	**MATERIALS AND METHODS**	40
	Preparation of Bacterial Culture	40
	P. aeruginosa as Biological Control Seed Treatment Agent on Tomato Seed	44
	Strain Identification	44
	Antagonism Towards *R. solanaceaum*	45

	Effects of Tomato Seed Bio-priming with *P. aeruginosa* On Percentage of Seed Germination	47
	Effects of Seed with *P. aeruginosa* Bio-priming on Tomato Plant Biomass	48
	Penicillium sp. as Biological Control Agent to Control Bacterial Wilt on Tomato Plant	49
	Antagonism of *Penicillium* sp. Towards *R. solanacearum*	52
	Efficacy of *Penicillium* sp. as Biocontrol Agent	53
	Effect of of *Penicillium* sp. Application on Biomass of Tomato Plant at Nursery Stage	54
	Effect of Calcium Nitrate Application on Plant Growth at Nursery Stage	56
	Greenhouse Bioassay	57
	Field Trial	67
IV	**RESULTS**	72
	Effect of the Application of *P. aeruginosa* as Biological Seed Treatment	72
	Antagonism of *P. aeruginosa* Towards *R. solanacearum*	72
	Effect of Tomato Seed Bio-priming with *P. aeruginosa* on Percentage of Seed Germination	78
	Effect of Seed Bio-priming with *P. aeruginosa* on Tomato Plant Biomass	81
	Application of *Penicillium* sp. as Biological Control Agent to Control Bacterial Wilt on Tomato Plant	83
	Antagonism of *Penicillium* sp. Towards *R. solanacearum*	83
	Efficacy of *Penicillium* sp. as Biocontrol Agent	83
	Effect of *Penicillium* sp. Application on Biomass of Tomato Plant at Nursery Stage	86
	Effect of Calcium Nitrate Application on Plant Growth at Nursery Stage	90
	Result of Greenhouse Bioassay	93
	Field Trial	100
V	**DISCUSSIONS**	111
	Antagonism of *P. aeruginosa* Towards *R. solanacearum*	111
	Effect of Application of *P. aeruginosa* as Biological Seed Treatment on Percentage of Seed Germination	114
	Effect of the Application of *P. aeruginosa* as Biological Seed Treatment on Plant Biomass	116
	Antagonism of *Penicillium* sp. to Against *R. solanacearum* and the Effect of the Application on Plant Biomass	119
	Evaluation of Biocontrol of Bacterial Wilt under Greenhouse	122
	Evaluation of Biocontrol of Bacterial Wilt under Field Condition	126
VI	**CONCLUSIONS**	131

BIBLIOGRAPHY 134

APPENDICES

A: Preparation of Media 157
B: Description of MT11 161
C: Monthly Weather Report 163
D: Chemical and Physical Properties of Field Soil 166
E: Table for the Percentage of Seed Germination after Bio-primed with *P. aeruginosa* 167
F: ANOVA Table 168
G: Commodity Price for Tomato (2001) 192

VITA 193

LIST OF TABLES

Table		Page
1	Recorded Hosts of *Ralstonia solanacearum* in Malaysia.	9
2	Antagonistic test of strains of *Pseudomonas aeruginosa* against the growth of *Ralstonia solanacearum* on King's B agar media.	76
3	Effect of seed bio-primed with different strains of antagonist *Pseudomonas aeruginosa* on mean biomass of tomato plants.	82
4	Effect of *Penicillium* sp. treatment on the plants' height and biomass of the top part of tomato seedlings.	88
5	Effects of calcium nitrate solution application on mean tomato plant's height, and biomass of the top part of seedlings at nursery stage.	91
6	Mean disease incidence of bacterial wilt on tomato after treatment with *Penicillium* sp. using different methods in the second greenhouse trial.	99
7	Mean disease incidence of bacterial wilt on tomato after treatment with *Penicillium* sp., at different levels of concentration in the second greenhouse trial.	99
8	Effect of the methods of application of biocontrol on the percentage of disease incidence on MT 11, eight weeks after transplanting in the field.	105
9	Conversion of yield and gross revenue per hectare and percent increase in revenue with treatment of MT11.	109

LIST OF FIGURES

Figure		Page
1	Bacterial slime that oozed out from the cut end of the stem of infected tomato when it was immersed in water.	41
2	Typical virulent bacterial colonies of *Ralstonia solanacearum* on tetrazolium chloride agar after 48 hours of incubation at 29 (± 1)°C.	43
3	Subculture of *Ralstonia solanacearum* on casamino acid peptone glucose agar after incubated at 29 (± 1)°C for 48 hours.	43
4	Culture of antagonist *Pseudomonas aeruginosa*, strain KT8 on King's B medium after incubation at 30 (± 1)°C for 24 hours.	46
5	Micrograph of *Penicillium* sp. from three-day-old culture on malt extract agar media, observed under magnification of 400 ×.	50
6	Colonies of *Penicillium* sp. on malt extract agar plate after incubation of 14 days at 25 (± 1)°C.	50
7	Colonies of *Penicillium* sp. on Czapek yeast autolysate agar plate after incubation for 14 days at 25 (± 1)°C.	51
8	Colonies of *Penicillium* sp. on Czapek yeast autolysate agar plate after incubation for 14 days at 35 (± 1)°C.	51
9	View of greenhouse experiment showing the arrangement of the plants.	59
10	Tomato plant (Disease Severity Index = 0 rating)	62
11	Tomato plant (Disease Severity Index = 1 rating)	63
12	Tomato plant (Disease Severity Index = 2 rating)	64
13	Tomato plant (Disease Severity Index = 3 rating)	65
14	Tomato plant (Disease Severity Index = 4 rating)	66
15	View of the field site showing the susceptible Pearl tomato and MT11 seedling just after transplanting from nursery.	69
16	A close-up of bacterium lysis in the culture of lysogenic strain, Eleu 4 after 24 hours of incubation at 30 (± 1)°C on	

	King's B medium.	73
17	Inhibition of the growth of *Ralstonia solanacearum* by antagonist *Pseudomonas aeruginosa* strains K2, KT8 and SC10/1 after incubation at 30 (± 1)°C for 48 hours on King's B agar media.	74
18	Inhibition of the growth of *Ralstonia solanacearum* by antagonist strain 72PTT on King's B agar media after incubation for 48 hours at 30 (± 1)°C.	74
19	The development of transparent outer layer around the colony grown from the mixture culture of strains SC10/1+ 72PTT on King's B medium after incubation of 48 hours at 30 (± 1 °C).	77
20	Translucent bacterial plaques were seen in the centre of the combination of strains, 72PTT+K2 after incubation of 48 hours at 30 (± 1 °C) on King's B media.	77
21	Effect of seed bio-priming with strains of *Pseudomonas aeruginosa* individually or in combination on the percentage of seed germination.	80
22	Inhibition of *Ralstonia solanacearum* culture by four-day-old antagonist *Penicillium* sp. on Malt Extract Agar media after incubation for 48 hours at 25 (±1)°C.	84
23	Inhibition of *Ralstonia solanacearum* culture by three-day-old antagonist *Penicillium* sp. on Potato Dextrose Agar after incubation for 48 hours at 25 (±1°)C.	84
24	Slight antagonism between combination of strains KT8+ 72PTT and *Penicillium* sp. on Malt Extract Agar after 48 hours of incubation at 30 (±1°)C.	85
25	Greenhouse evaluation of efficacy of *Penicillium* sp. to control the incidence of bacterial wilt on tomato plants at the nursery stage.	85
26	Incidence of bacterial wilt on tomato plants treated with different concentration of *Penicillium* sp. at the nursery stage.	87
27	Effect of different method application of antagonist *Penicillium* sp. culture on the mean biomass of tomato plants.	89
28	Tomato seedlings were treated with calcium nitrate solution in different concentration in greenhouse experiment for four	

	weeks.	92
29	Disease Severity Index of bacterial wilt on MT11 after treatments with *Pseudomonas aeruginosa* and *Penicillium* sp. in first greenhouse trial.	94
30	Disease Severity Index for bacterial wilt disease on MT11 after treatments with *Pseudomonas aeruginosa* and *Penicillium* sp. in second greenhouse trial.	95
31	Mean percentage of disease incidence on tomato after treatments with *Pseudomonas aeruginosa* and *Penicillium* sp. from two greenhouse trials, four weeks after transplanting.	98
32	Mean Disease Severity Index (DSI) of bacterial wilt of two varieties of tomato with treatments of *Pseudomonas aeruginosa* and *Penicillium* sp. in field trial.	101
33	Field trial view showed the susceptible Pearl tomato almost wiped out by bacteria wilt compared to MT11 plants.	102
34	Progress of means percentage of disease incidence of bacterial wilt on different variety of tomato plants after treatments with *Pseudomonas aeruginosa* and *Penicillium* sp.	104
35	Size of ripe tomatoes (MT11) from field plantation.	107
36	Means of fruit yield from different variety of tomato plants with treatment of biocontrol agent in field trial.	108

LIST OF ABBREVIATIONS

ANOVA	=	Analysis of Variance
$Ca(NO_3)_2$	=	Calcium nitrate
cfu	=	colony forming unit
CPG	=	casamino acid peptone glucose
CYA	=	Czapek's yeast autolysate
DMRT	=	Duncan's Multiple Range Test
DSI	=	Disease Severity Index
FAMA	=	Federal Agriculture and Marketing Authority
FAO	=	Food and Agriculture Organization
ISR	=	Induced Systemic Resistance
K	=	potassium
KB	=	King's B
LSD	=	Least Significant Difference
MARDI	=	Malaysian Agricultural Research and Development Institute
MEA	=	malt extract agar
N	=	nitrogen
OD	=	Optical density
P	=	phosphorus
PDA	=	potato dextrose agar
PGPR	=	Plant Growth-Promoting Rhizobacteria
pH	=	Hydrogen ion concentration
TZC	=	tetrazolium chloride
Vol.	=	volume

CHAPTER I

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the economically important vegetables throughout the world. In Malaysia, the planting hectareage and production of tomatoes has rapidly increased during 1989 to 1999 (FAO, 2000). It is the third important vegetable of the export commodities of Malaysia. It has become a potential source of cash income for the farmers. Recent interest has been directed towards the red pigment in the ripe tomato fruit, lycopene, one of the world's most powerful antioxidants (Jones, 1999). In Malaysia, production of tomato tends to be more successful in highland area, primarily because of the mild temperature. Disease and pests often hamper the tomato production. Occurrence of tomato disease can cause the reduction in the quality and quantity of the yield.

The bacterial wilt disease caused by *Ralstonia solanacearum* (syn. *Pseudomonas solanacearum*)(Yabuuchi *et al.*, 1992) was considered as the most serious factor limiting commercial production in the lowlands (Graham *et al.*, 1977). Therefore large-scale tomato cultivation was presently carried out in Cameron Highlands. In Malaysia, this soil-borne pathogen had been reported on 43 hosts distributed over a wide range of families which, includes crop plants, ornamentals and weeds (Abdullah, 1992). It also includes some major economic hosts, such as brinjal, chilli, ginger, groundnut, potato, tobacco and tomato. Losses due to this disease had been reported from cultivated or non-cultivated plants from many countries of the world.

However, the estimates of losses in tomato production in Malaysia due to bacterial wilt disease are not available.

Control measures commonly employed to control bacterial wilt include resistant or tolerant varieties or cultivars, crop sanitation, crop rotation and other cultural practices. Greenhouse experiments showed that chemical control measures were able to significantly reduce the incidence of bacterial wilt in chilli and tomato plant (Abdullah, 1998). The use of resistant varieties is known as the most effective, popular and easiest strategy for disease control. However, for controlling bacterial wilt disease, resistance is not expressed under certain environmental conditions (Sequeira and Rowe, 1969) and the breakdown of resistance due to high temperature has been known to occur (Krausz and Thurstan, 1975; French and De Lindo, 1982).

Biological control, a promising control strategy has provided an alternative for the management of bacterial wilt. Many researchers have investigated the introduction of antagonistic microbial agents in biological control during the past few years (Chen and Echandi, 1984; Ciampi *et al.*, 1989; Trigalet and Demery, 1990; Hara and Ono, 1991). Strains of fluorescent pseudomonad have been reported as beneficial microbial agent to increase growth and control soilborne plant pathogens (Schroth and Hancook, 1982; Suslow, 1982; Burr and Caesar, 1984; Leeman *et al.*, 1995). Some of them are known as plant-growth promoting rhizobacteria (Kloepper *et al.*, 1980b; Ongena *et. al.*, 1999). Identification of these fluorescent pseudomonads is readily made by the production of pigmented siderophores, which fluoresces under ultra violet and blue light (Kloepper *et al.*. 1980a).

Even though there was no report of the control of bacterial wilt with *Penicillium* sp., it has been reported as a biocontrol agent to control other diseases (Windels, 1981; Kaiser and Hannan, 1984; Fang and Tsao, 1995; De Cal *et al.*, 1995, 2000). To date, there is no effective control measure to control bacterial wilt disease in heavily infested areas in Malaysia. A new approach in the management of this disease using moderately susceptible variety coupled with microbial antagonists and calcium nutrient amendment was investigated for their effectiveness. The objectives of the study were:

1. To determine the effect of bio-priming with *Pseudomonas aeruginosa* on tomato seed germination and the biomass of plant.
2. To evaluate the effect of application methods of the microbial antagonists, in controlling bacterial wilt of tomato under greenhouse and field conditions.